IDENTIFICATION OF NOVEL INHIBITORY PEPTIDES OF PROTEIN-PROTEIN INTERACTIONS INVOLVED IN DNA REPAIR AS POTENTIAL DRUGS IN BREAST CANCER TREATMENT

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Protein-protein interactions are critical to almost every cellular process. Disruption of these interactions would effectively interfere with the cell's functions and its ability to grow and divide normally. Most chemotheraputic drugs target DNA and cause extensive double strand breaks. Double strand breaks are repaired by two major pathways – Homologous Recombination (HR) and the Non Homologous End Joining (NHEJ). Disrupting these pathways is thus an attractive target for small molecules to be used as sensitizing agents. Deficiency in DNA damage repair should sensitize cells to DNA damaging agents and thus such tumors could be effectively treated with a lower dose of chemotherapeutic agents/radiation. The Rad51 and Rad52 proteins are important proteins involved in HR. Rad51 acts as a hexamer that binds single-stranded DNA to drive strand exchange during HR. The Ku70-Ku80 dimeric complex is the primary damage sensor in NHEJ. We are targeting these two complexes (RAD51 multimer and Ku70-Ku80 dimer) for disruption using several means.

In one approach we are using a plasmid library encoding for 12-15 amino acid peptides to screen for inhibitory peptides for these complexes because it is known that short peptides of 5-10 amino acids can destabilize protein-protein interactions. We are using the yeast reverse two-hybrid system for this screen (M Vidal et al, PNAS, 93:10315, 1996).

We are also using in silico methods to identify pockets on the protein surface that are attractive targets to dock inhibitory compounds. These pockets are identified by looking for cavities on the surface of the complex using the CAST software (J Liang et al, Prot Sci, 7:1884, 1998), mapping the interaction surfaces of the two chains, and then identifying surfaces that are accessible to the solvent and also have potentially important interactions for dimerization. We have thus far identified two regions of the Ku70-Ku80 complex, designated Pocket 154 and Pocket 157. We will report on our efforts to identify and target important sites on these protein complexes, disruption of which would sensitize breast cancer cells to chemotherapeutic agents.

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DRUG DISCOVERY FOR BREAST CANCER BY MIRROR-IMAGE LIGAND DISPLAY

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The goal of these experiments is to identify degradation-resistant ligands that bind to a breast cancer-specific epitope of the DF3 protein product of the MUC-1 gene. The proposed approach was to identify from a phage display library L-peptides that bind to the mirror image D-form of the native MUC-1 epitope. By the laws of stereochemistry, the D-enantiomer of the selected peptide should bind identically to the native L-enantiomeric form of the MUC-1 target. D-peptide ligands have the same chemical diversity as L-peptides, but with the greatly improved pharmacokinetic profiles needed for drug activity.

The mirror image (D-peptide) synthetic target was an oligomeric form of the 20 amino acid MUC-1 epitope, containing three tandem copies of the repeat (60 residues). This polypeptide includes the minimum number of repeats necessary to maintain a stable structure, and enables the possibility of selecting multivalent ligands which may have much higher target affinity.

The 60-residue D-peptide, with a biotin group coupled to the N-terminus, was chemically synthesized, and used as "bait" for two random peptide libraries using phage display. The first library screened was a random hexapeptide library, and the second library contained a 10 residue random insert flanked by ser/cys at either end. S-protein, for which a consensus sequence of selected peptides is well-known, was used as a control. Clear consensus sequences emerged from the phage libraries after biopanning against the S-protein control. In contrast, neither library led to identification of a consensus with the MUC-1 target. We thus conclude that mirror-image phage display failed to identify a peptide that binds to the D-MUC-1 target amino-acid sequence.

One explanation for the failure of phage display to yield a ligand for the MUC-1 epitope is that the library size (10E8) is too small. Thus, we are adapting the approach of ribosome display to increase the library size by several orders of magnitude. In developing a platform for presenting ribosome display libraries (which are not commercially available), we have reconstituted peptide synthesis from purified components. This approach will allow us to resume the search for drug candidates that bind to the breast-cancer specific MUC-1 epitope.

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BENZOPYRANONE DIVERSITY AND BIOACTIVITY FOR MOLECULAR TARGETS IN BREAST CANCER

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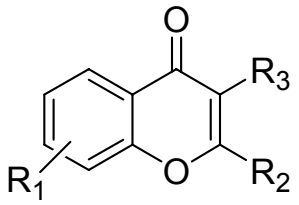
An estimated 60-70% of human breast cancers are hormone-dependent and require estrogen for growth. Our research focuses on the development of medicinal agents can alter estrogen biosynthesis and estrogen action, therefore influencing breast tumor development and growth. Selective steroidal and nonsteroidal agents are effective in inhibiting breast tissue aromatase and/or in blocking the estrogen receptor. The benzopyranone ring system is a molecular scaffold of considerable interest, and this scaffold is found in isoflavonoid natural products that have weak estrogen agonist or antagonist activity and in flavonoids that exhibit weak aromatase inhibitory activity.



Benzopyranones

Our medicinal chemistry efforts focus on diversifying the benzopyranone scaffold and on utilizing combinatorial chemistry approaches to construct small benzopyranone libraries as potential therapeutic agents. Benzopyranone compounds in our libraries were evaluated in biological studies examining their effects on human breast cancer cell lines. Bioassays include cell proliferation in MCF-7 (ER+) and MDA-MB231 (ER-) human breast cancer cell lines. Differential activities were observed with clusters of structurally similar compounds, and the analysis identified several agents exhibiting enhanced activities on breast cancer cell growth. Estrogen receptor affinities were determined by radioligand displacement assay using cytosolic rat uterus estrogen receptor extract. A few compounds did show some binding activity, with activities of 50% to 67% of genistein (a positive control). Several compounds in the initial libraries demonstrated moderate aromatase inhibitory activity in human placental microsomal assays, with IC_{50} values ranging from 1 to 30 μM. The results of these studies are being used to develop computational molecular models for binding of the compounds to key macromolecules.

The initial combinatorial library of synthetic benzopyranones has resulted in agents exhibiting enhanced and differential activities on breast cancer cell growth, estrogen receptor affinity, and aromatase inhibition. Synthetic efforts are continuing and focus on the development of more selective agents for molecular targets in breast cancer. The design, synthesis, and screening of substituted benzopyrone combinatorial libraries are enabling us to explore the biological potential of these molecules and develop more selective agents for critical targets in breast cancer.

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DEVELOPMENT OF CHEMICAL AND GENETIC SYNTHETIC LETHALITY SCREENS IN BRCA1-DEFICIENT CULTURED TUMOR CELLS: A NOVEL APPROACH TO ANTICANCER DRUG DISCOVERY

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The mechanism-based anticancer drug discovery approach is currently concentrated for the most part on either inhibiting the overexpression of key dominant oncogenes or reconstructing the crippled activity of tumor suppressor genes. A different approach is to examine whether these primary tumor-specific alterations sensitize the malignant cell to drugs aimed at secondary targets, which may have either normal or abnormal expression in the tumor context. In the case of familial breast cancer with mutations in the breast cancer susceptibility gene BRCA1, the question is whether this particular gene deficiency can be turned into the Achilles heel of this tumor by finding drugs or mutant genes which would synergize with BRCA1-deficiency to cause tumor-specific cell death.

Previously, we established chemical and genetic synthetic/synergistic lethality screens in cultured human cells, based in part on the concept of the yeast genetic method [Simons et al., (2001) Genome Res. 11, 266-273; Simons et al., (2001) Nucleic Acids Res. 29, e100; respectively]. In this project we are applying the indicated technologies to human cells deficient in BRCA1, the primary gene of interest. These cells are complemented by an episomal "survival plasmid" expressing the BRCA1 cDNA, while harboring a novel GFP-based double-label fluorescence system. Selective pressure imposed by chemicals conferring double strand breaks should prevent the spontaneous loss of the episomal "survival plasmid." Retention or loss over time of the "survival plasmid" will be detected by measuring the fluorescence levels expressed off the GFP-tagged "survival plasmid." The results of this line of investigation should clarify the feasibility of high throughput screening for drugs synthetically lethal with BRCA1 deficiency in these cells.

In the second phase of this project, we seek to identify genes which, in their mutant form, are synthetic/synergistic lethal with BRCA1-deficiency. Dominant-negative Genetic Suppressor Elements (GSEs) for synthetic lethal genes selected from a transfected library expressing short, truncated sense and antisense cDNAs should prevent the spontaneous loss of the episomal "survival plasmid," to be followed by isolation of cells retaining the "survival plasmid" and the rescue of the putative GSEs. The dominant-negative nature of the GSEs would be validated by independent means. The importance of this research stems from its focused search for drugs and mutant genes which are not directed against the primary BRCA1 lesion, but rather towards secondary targets sensitized by the presence of the BRCA1 deficiency. As such, this novel approach should significantly broaden the spectrum of drugs/targets directed towards BRCA1-related breast cancer.

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USING GSTP1-1 TO GENERATE CYTOTOXIC EXOCYCLIC ENONES IN MDR BREAST TUMORS

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The *Streptomyces* metabolite 2-crotonyloxymethyl-(4R,5R,6R)-4,5,6-trihydroxy-2-cyclohexenone (COTC) and its synthetic analog 2-crotonyloxymethyl-2-cyclohexenone (COMC-6) are known to be potent antitumor agents for both murine and human tumors in culture and in tumor-bearing mice. Early investigators proposed that antitumor activity might arise from competitive inhibition of the methylglyoxal detoxifying enzyme glyoxalase I by the covalent adducts arising from the S_N2 displacement of crotonate by intracellular glutathione (GSH). However, this hypothesis is no longer tenable, as the GSH adducts of COTC and COMC-6 are poor competitive inhibitors of human erythrocyte glyoxalase I (K_is 100-200 μM), and the GSH adduct of COMC-6 (GSMC-6) did not exhibit significant antitumor activity toward melanotic melanoma in tissue culture, when delivered into these cells as the [*glycyl, glutamyl*] diethyl ester prodrug. This is in contrast to the potency of COMC-6 (IC_{50} = 0.041 μM) and its five and seven membered ring homologues COMC-5 (IC_{50} = 0.14 μM) and COMC-7 (IC_{50} = 0.029 μM) in these cells. We now propose an alternative hypothesis for antitumor activity, on the basis of the discovery that human placental glutathione transferase (GSTP1-1) catalyzes the conjugation of COMC-6 with GSH. Kinetic studies and intermediate trapping experiments show that this is a multistep process first involving enzyme-catalyzed addition of GSH to COMC-6 to give a highly reactive exocyclic enone product, which subsequently reacts with GSH in bulk solvent to give GSMC-6. Molecular docking studies using the X-ray structure of the human GSTP1-1 isozyme suggest that Tyr 108 functions as a general acid catalyst by H-bonding with the carbonyl oxygen of bound COMC-6 during the anti-addition of GSH. In principle, hGSTP1-1 might play a key role in the antitumor activity of these compounds, particularly in MDR cells overexpressing GST. In principle, the GST-generated exocyclic enones could react with proteins and/or nucleic acids critical to cell function, thus, inhibiting the cells. Indeed, this hypothesis is supported by mass spectral studies indicating that COMC-6 alkylates model oligonucleotides in the presence of GSH via a mechanism in which the exocyclic enone is probably the alkylating species.

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1-ALPHA HYDROXYVITAMIN D5 AS A POSSIBLE CHEMOTHERAPEUTIC AGENT

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Recently, we identified a novel vitamin D analog, 1α-hydroxy-24ethyl vitamin D5 (1α(OH)D5). In this CTR application, we evaluated the possible therapeutic effect of 1 α (OH)D5 for breast cancer. We also performed preclinical toxicity studies to determine whether vitamin D analog is safe and nontoxic. We showed earlier that 1α(OH)D5 had a chemopreventive effect on experimental breast cancer. It prevented development of carcinogen-induced precancerous lesions in mouse mammary gland organ culture. In this study, we focused on the therapeutic property of the compound. 1α(OH)D5 has both growth-inhibitory and cell-differentiating actions in breast cancer cells. Treatment of VDR+ breast cancer cells with 1 μM 1α(OH)D5 resulted in dose-dependent cell growth inhibition. However, VDR- cells were non-responsive to 1α(OH)D5. These results suggested that the effect of 1α(OH)D5 was in part mediated by VDR. In selected cell lines, 1α(OH)D5 induced cell cycle arrest in the G1 phase. In addition, analog treatment also induced cell differentiation in breast cancer cells. Following 7 days treatment with 1α(OH)D5, we observed increased accumulation of beta casein and neutral lipids in the cytoplasm and increased expression of cell surface alpha2 integrin. Preliminary microarray gene cluster analysis showed that, following incubation of breast cancer cells with 1α(OH)D5, selective down regulation occurs of estrogen-inducible genes such as PS2 and PR. These results were further confirmed by RT-PCR. In order to translate our results obtained in cells to human tissues, we studied in vitro effects of vitamin D analog on normal and malignant breast tissues obtained from women with confirmed diagnosis of breast cancer. 1α(OH)D5 had no effect on nonmalignant breast epithelial cells, but it induced apoptosis in breast cancer. It also showed apoptotic effect in fibroadenomas. In order to confirm the safety of the vitamin D analog, we performed detailed preclinical toxicity studies in two different species, rats and dogs, under GLP regulations. The maximum tolerated dose for rats is 100 μg/kg body weight, whereas dogs are more sensitive to 1α(OH)D5 and can tolerate it at a safe dose of D5 μg/kg body weight. We are currently in the process of filing an IND application.

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CELL-CYCLE CONTROL BY NATURAL AND SYNTHETIC INDOLE-3-CARBINOL-RELATED COMPOUNDS ON HUMAN BREAST CANCER CELLS

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Differences in the diet are thought to be one of the critical environmental components that has contributed significantly to the wide variety of breast cancer rates in various world populations. These epidemiological findings suggest that dietary plants produce specific biologically active compounds that represent a potential source of new therapeutic medicines for the control breast cancer. One such naturally occurring phytochemical from Brassica vegetables, such as cabbage, broccoli, and Brussels sprouts, is indole-3-carbinol (I3C), which has been shown to be an effective chemopreventive agent in a variety of tumor models. We have documented that I3C arrests the growth of cultured human breast cancer cells by inducing a G1 cell cycle arrest independent of estrogen receptor function. The I3C dependent cell cycle arrest is accompanied by the selective and rapid inhibition in expression of the G1-acting cyclin dependent kinase 6 (CDK6) transcripts and protein, down regulation of CDK6 promoter activity, and inhibition of CDK2 specific enzymatic activity. We have shown that I3C synergizes with tamoxifen to arrest the growth of estrogen responsive human breast cancer cells. Given the limited information about the potential role of indoles in the treatment breast cancer, a necessary first experimental step for the development of novel I3C-based therapeutic compounds, is to uncover I3C derivatives that have a more potent growth inhibitory effect than I3C itself. We have initiated a structure-activity analysis of the anti-proliferative effects of I3C using both natural and synthetic I3C compounds. Analysis of cell cycle control by flow cytometry expression of CDK6 transcripts and inhibition of CDK2 enzymatic activity revealed several I3C-based compounds with more potent anti-proliferative effects than I3C. These I3C derivatives work similarly to I3C but at lower concentrations. Moreover, using a similar cell cycle analysis, the anti-proliferative activities of several other natural I3C-based indole compounds were found to be ablated. This work is being extended in cell culture experiments to uncover more potent I3C derivatives, and to test the anti-tumor effects of the most promising I3C derivatives, alone or in combination with tamoxifen, on the in vivo

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(+)-DISCODERMOLIDE: A POTENTIALLY IMPORTANT ANTITUMOR AGENT

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An efficient, highly convergent, stereocontrolled total synthesis of the potent antimitotic agent (+)-discodermolide, a rare marine sponge metabolite possessing extraordinary anticancer activity, has been achieved on gram scale. Key elements of the successful strategy include: (1) elaboration of three advanced fragments from a common precursor, which embodies the repeating stereochemical triad of the discodermolide backbone; (2) installation of the Z trisubstituted olefin, exploiting a modified Negishi cross-coupling reaction; (3) synthesis of a late-stage phosphonium salt utilizing high pressure; and (4) Wittig installation of the Z disubstituted olefin and the terminal Z diene.

With access to (+)-discodermolide, the solution structure of (+)-discodermolide was determined via 1 and 2-D NMR techniques in conjunction with Monte Carlo conformational analysis. Taken together the results demonstrate that in solution (+)-discodermolide occupies a helical conformation remarkably similar to the solid state conformation. In addition an overall strategy for the design, synthesis, and biological evaluation of synthetic analogs of (+)-discodermolide will be presented.

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THE ROLE OF SYMBIOTIC BACTERIA IN THE BIOSYNTHESIS OF BRYOSTATINS: INVESTIGATION OF CULTIVATION OF A BRYOSTATIN-PRODUCING BACTERIAL SYMBIONT OF THE MARINE BRYOZOAN *BUGULA NERITINA*

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Bryostatin 1 is an anticancer drug in Phase I and II clinical trials. It appears to be particularly useful in combined therapy since it acts synergistically with other drugs and can reverse multidrug resistance. In animal studies, it also shows particular promise in adoptive immunotherapy for breast cancer.

Bryostatins are extracted from the marine bryozoan Bugula neritina. Bryostatins are present in low amounts, making research and drug development difficult. Bryostatins are similar to compounds produced by bacteria. Our lab has been addressing the hypothesis that bryostatins are biosynthesized by symbiotic bacteria in the host bryozoan. We have obtained strong evidence for involvement of the symbiont, "Candidatus Endobugula sertula" in the biosynthesis of bryostatins and cloned a putative bryostatin biosynthesis cluster (see L. Waggoner poster). If the symbiont could be cultivated, or the biosynthetic pathway expressed in an industrial host, bryostatins could be produced in abundance allowing their development as drugs for treatment of breast cancer.

To cultivate the symbiont we need to satisfy several prerequisites. First, we need a inoculum with abundant E. sertula cells and few other bacteria; these cells must be viable. Second we need a sensitive and specific method for detecting the cells in a high throughtput format to allow comprehensive cultivation studies. We have developed a protocol for isolating E. sertula cells with >95% purity from B. neritina larvae using Percoll density gradients. Viability of these cells is still under investigation. We have developed an assay based on in situ hybridization of specific rRNA oligonucleotide probes, but would like to achieve greater sensitivity and reliability. We are currently exploring bacterial chromosome painting to achieve this. When we have completed development of our assay we will begin large-scale cultivation studies. Preliminary small-scale cultivation experiments have helped us to refine our techniques, but have not yielded an E. sertula isolate.

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MORPHOMETRIC ASSAY FOR MICROTUBULE INHIBITOR COMBINATION DRUG EFFECTS

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One problematic aspect of drug development is that assays for in vitro or in vivo effects in model systems have limited ability to predict clinical drug efficacy. We have developed an assay for cell shape phenotypes, which is based on extracting interference contours from cells that are grown on solid substrate interferometers. Values for 102 variables are computed. Their values describe the shape of the contours and comprise a unique description of each cell. Using standard classification techniques, we found that a "cancer-type" cell has common features, even in lines originating from different tissues. The finding that cancer-type reversal occurs in cells treated with paclitaxel (PX) and colchicine (CC) led to our original proposal that the inhibitor combination (IC) be tested as a clinical chemotherapy. Some variables that are changed in response to this IC are correlated with values of a factor, #4. (Factors are theoretical variables based on the covariance of the variables' values.) Factor #4 quantifies filopodia at the cell edge and accounts for the biggest single difference between normal and cancer cells. These features are regulated by a Rho-family GTPase, Cdc42, that is activated downstream of Ras. Other taxanes and ICs were evaluated to see whether they caused similar changes in variables underlying #4. While ICs based on cephalomannine and 7-deoxytaxol had no such effect, cells treated with 7-deoxytaxol or cephalomannine alone showed reversal in two or one of the variables compared to the direction they changed in cancer-type cells, respectively. Cephalomannine alone and an IC based on 7-deoxytaxol affected factor #7, which reflect a morphological feature called stress fibers. These structures, normally regulated by another GTPase RhoA, are enhanced in cancer-type cells. The data suggest that enhancement of this feature in treated cells counteracts the trend to exhibit cancer-type reversal. The effects of a portmanteau drug made from PX and CC, as well as other microtubule-depolymerizing agents in ICs with PX, are being tested. The work suggests that assays for cancer-type reversal will predict whether a compound or IC has potential as an antipromoter or chemotherapeutic agent, and that such assays serve as a guide to drug development.

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DELIVERY OF STREPTAVIDIN CONJUGATES INTO BREAST CANCER CELLS MEDIATED BY A NOVEL SMALL-MOLECULE LIGAND

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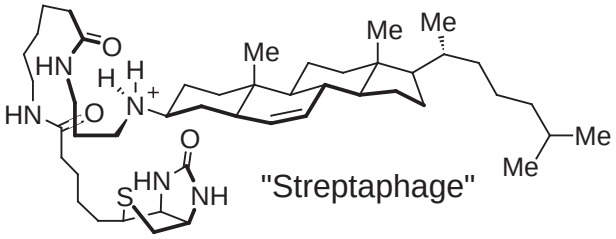
The efficient delivery of macromolecules into tumor cells presents a formidable challenge to the development of effective macromolecular anticancer therapeutics. Although numerous methods for intracellular delivery of macromolecules to cancer cells have been reported, the molecular mechanisms underlying many of these methods are not well understood. As a consequence, efficiencies of macromolecular delivery are often limited by variability, toxicity, and unpredictable cell-type specificity. Hence, novel methods that enhance uptake of weakly permeable molecules are needed in basic cell biology, tumor therapy, and genetic therapy.

We describe here a novel synthetic ligand termed Streptaphage that promotes cellular uptake of the bacterial protein streptavidin by promoting non-covalent interactions with lipid raft subdomains of cellular plasma membranes. The ligand comprises an N-alkyl derivative of 3β-cholesterylamine linked to the carboxylate of biotin through an 11-atom tether. Molecular recognition between fluorescent conjugates of streptavidin and this ligand at plasma membranes promotes clathrin-mediated endocytosis, which renders streptavidin partially intracellular within 10 minutes and completely internalized within 4 hours of protein addition. Analysis of protein uptake in Jurkat lymphocytes revealed intracellular fluorescence enhancements at 10 μM ligand of over 300-fold with > 99% efficiency and low toxicity. Importantly, MCF-7 breast cancer cells were four-fold more susceptible than Jurkat lymphocytes to ligand-regulated delivery of streptavidin though this mechanism.



"Streptaphage"

Multidrug resistant (MDR) breast cancers are known to dramatically overproduce components of lipid rafts, and these cell lines may be particularly susceptible to this delivery strategy. Recent efforts directed at ligand-regulated delivery of toxins conjugated to streptavidin to MCF-7 breast cancer cells and multidrug resistant (MDR) breast cancer cell lines will be described.

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LIPOSOMAL-DIMETHYL-SPHINGOSINE AND PACLITAXEL COPOLYMER ARE ACTIVE AGAINST HER-2/NEU-OVEREXPRESSING HUMAN BREAST ADENOCARCINOMA ORTHOTOPIC XENOGRAFT MODEL

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Over-expression of HER-2/neu has been linked to poorer prognosis and survival in breast cancer patients. The basis for this association likely includes therapeutic resistance, including resistance to Taxol (paclitaxel), widely used in many chemotherapeutic regimens for this disease. We have recently observed that certain sphingolipids, either as free lipids or as constituents of liposomes, induce apoptosis in vitro in tumor cells despite the over-expression of Her-2/neu. Further, we have reported that a paclitaxel copolymer, paclitaxel-poly(L-glutamic acid) (PGA-TXL), is active against Taxol-resistant tumors in vivo.

We therefore evaluated liposomal-dimethyl-sphingosine (L-DMSP) and PGA-TXL in a human HER-2/neu over-expressing breast adenocarcinoma (MDA-361) orthotopic xenograft model. Tumor cells (4-6 X 106) were implanted in the mammary fat pad of 5-8 week old female nude mice. Mice were treated i.p. either one-week later or when tumors grew to 5-6 mm diameter.

Early treatment with a multiple-dose regimen of L-DMSP (4.5 mg DMSP per dose; 20 mole percent of a small unilamellar vesicle formulation), caused a delay in or reduced subsequent tumor growth, but was not curative. However, early treatment with a single-dose of PGA-TXL (180 mg/kg paclitaxel equivalents), also one week after tumor implantation, resulted in substantial tumor growth delay, regression, or even apparent cure in two of four mice (control tumor areas at 10 weeks post-implant = 44 _ 21.2 mm2 ; treated group areas = 6 _ 6.0 mm2). When administered at the later timepoint to another group of animals, PGA-TXL still caused tumor growth delay, but no cures were observed (treated group areas = 24 _ 15.3 mm2); nor did administration of L-DMSp at this time appear to be efficacious.

We conclude that DMSP as a liposomal formulation has some efficacy against this HER-2/neu over-expressing model when the tumor burden is low. Formulation of paclitaxel with the poly(L-glutamic acid) backbone substantially reduced its toxicity, enhanced its potency, and rendered it active against this HER-2/neu over-expressing breast adenocarcinoma model.

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NEW NAPHTHOQUINONE SPIROKETALS WITH ANTIPROLIFERATIVE ACTIVITY AGAINST HUMAN BREAST CANCER CELLS

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We are testing the hypothesis that the thioredoxin redox signaling system is essential for the growth of some human breast cancers and that drugs inhibiting this system will block oncogenesis and cause selective growth inhibition and/or apoptosis. Our specific objectives were to generate and identify selective inhibitors of thioredoxin using target-array chemistry methodologies, in vitro assays and cell-based screening approaches. Our initial studies revealed members of the naphthoquinone spiroketal natural product family, which includes preussomerin, palmarumycin and diepoxin, inhibited thioredoxin in vitro. Because these natural products are of considerable synthetic interest, we develop semi-automated synthetic methodologies to generate a targeted library for further study. We evaluated 22 naphthoquinone spiroketals for inhibition of mammalian thioredoxin, thioredoxin-reductase, and breast cancer cell growth. Several of these novel structures were effective against human MCF-7 and MDA-MB-231 cells with median growth inhibition (IC50) values in the low or submicromolar range. Moreover, we discovered a new nanomolar lead structure for the development of inhibitors of the thioredoxin-thioredoxin reductase system. Palmarumycin CP1 rivaled the enzyme inhibitory activity reported for the structurally much more complex and more electrophilic quinone pleurotin and showed 50% growth inhibition at 10-20 times lower concentrations. Our structure activity relationship studies on the synthetic derivatives demonstrated that both the phenol group and the enone moiety in the palmarumycin CP1 were important for maximizing enzymatic activity and that the presence of the naphthalenediol ketal enhances thioredoxin over thioredoxin reductase selectivity. Thus, the palmarumycins represent an attractive new opportunity for the development of new agents directed against human breast cancer.

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BREAST CARCINOMA CELL-TARGETED THERAPY BY NOVEL VITAMIN D ANALOG

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Vitamin D and its analogs have both growth-suppressing and cell-differentiating actions in various neoplastic cell types. However, their use as therapeutic or chemopreventive agents is hindered due to their high calcemic activity. In this study, we present a new vitamin D analog. 1α hydroxy D5 (D5) belongs to the D5 series in vitamin D classification. D5 was evaluated as a potential therapeutic agent for breast cancer. In vitro, following 7 days incubation of breast cancer cells with D5, we observed increased accumulation of intra-cytoplasmic droplets of neutral lipids, cell surface intracytoplasmic accumulation of beta casein, and increased expression of cell-surface expression of the alpha2 integrin. The effect of D5 in vitro was dose-dependent. The optimum cell-differentiating effect was observed at 1 μM concentration. In vitro, D5 (at 1 μM dose) also showed a growth-inhibitory effect on selected breast cancer cell lines. Breast cancer cells pre-incubated in vitro with D5 (at 1 μM concentration) failed to form tumors when transplanted into athymic mice. In vivo, dietary supplement of D5 (12.5 μg/kg body weight) significantly ($p<0.05$) inhibited the growth of BT-474 cells transplanted into athymic mice. Dietary supplement caused small and insignificant increase in serum calcium levels.

We further evaluated whether targeted delivery of D5 is effective and safe. D5 was linked to Her-2 antibody using SANPAH as a linker. Her-2 antibody used in this study had no significant effect on the growth of breast cancer cells but had some differentiating action, as evident from the intracellular accumulation of neutral lipids in BT-474 cells. Her-2 antibody-D5 immunoconjugate showed specific binding to Her-2-expressing breast cancer cells, competed with Her-2 antibody for surface receptor, and showed intracellular internalization. In vivo, i.p. administration of immunoconjugate significantly ($p<0.05$) reduced the growth of breast cancer cells transplanted into athymic mice. Tumor growth inhibition observed following immunoconjugate was similar to that observed in animals given dietary supplement of D5. Immunoconjugate treatment did not change serum calcium levels, and serum calcium was similar to that observed in animals treated with vehicle control.

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NATURAL PRODUCTS THAT TARGET PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-GAMMA (PPAR-GAMMA) IN BREAST TUMOR CELLS

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The focus of this project is to identify new activators of the nuclear hormone receptor/transcription factor, Peroxisome Proliferator-Activated Receptors-gamma (PPAR-gamma). PPARs have recently been identified as important new targets for anticancer drug discovery. Activation of PPAR-gamma can inhibit growth, cause terminal differentiation, and induce apoptosis in human breast tumor cells. Most currently known PPAR-gammactivators are fatty acid metabolites or structurally related to the synthetic thiazolidindione (TZD) class of insulin sensitizers. Marine algae and invertebrates produce hundreds of chemically diverse fatty acid metabolites found nowhere else in nature. These molecules, called oxylipins, include lipoxygenase metabolites known to activate PPAR-gamma, as well as structural analogs of endogenous human PPAR-gamma ligands (hydroxy-eicosanoids and prostaglandins).

Extracts and purified compounds from plants, marine invertebrates and algae have been examined for the ability to activate PPAR-gamma in breast tumor cell-based reporter assays. Briefly, a luciferase reporter under the control of the PPAR-gamma response element from the fatty acid binding protein (aP2) gene was used to monitor the activation of PPAR-gamma in MCF-7 breast tumor cells in vitro.

Structurally distinct hydroxylated and carbo-cyclized oxylipins and unusual marine lipopeptides produced by marine cyanobacteria and algae act as activators of PPAR-gamma. Triterpene acids produced by plants were found to activate PPAR-gamma in MCF-7 cells. The activation of PPAR-gamma in MCF-7 cells by marine oxylipins and triterpene acid is associated with reduced cell viability, possibly through induction of apoptosis. Several series of other marine natural products, unrelated to all other known PPAR-gamma activators act as partial agonists of PPAR-gamma.

The biochemical diversity represented by secondary metabolites produced by plants and marine organisms offer new and structurally diverse prototypes for the discovery of novel activators of PPAR-gamma. These substances may provide new insights into the structure and function of PPAR-gamma in tumorigenesis.

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NATURAL PRODUCTS THAT TARGET HYPOXIC BREAST TUMOR CELLS

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The objective of this project is to discover novel chemotherapeutic agents that target hypoxic breast tumor cells. Hypoxia (low oxygen) has been known as a factor that contributes to treatment resistance against ionizing radiation and some cytotoxic drugs, increases metastasis, and accelerates malignant progression in breast cancer. Although one hypoxia-activated cytotoxin (tirapazamine) is in clinical trial, no drug that directly targets hypoxic tumor cells is in clinical use. Drugs that selectively target treatment-resistant hypoxic tumor cells will dramatically improve current treatment options and enhance the outcomes.

To discover hypoxia selective drugs, we take a unique approach that combines traditional natural product drug discovery with in vitro bioassays that model the in vivo pathophysiological conditions of hypoxic breast tumors. High-throughput assays for inhibitors of hypoxia inducible factor 1 (HIF-1) activation have been developed in human breast tumor cells. HIF-1 is a hypoxia induced transcription factor that activates expression of genes that are required for tumor cells to adapt and survive in a hypoxic environment. HIF-1 activity is monitored using a luciferase reporter gene that is under the control of HIF-1 response elements. Hypoxic activation of HIF-1 leads to increased transcription from the reporter gene and results in increased luciferase activity. Both crude extracts and pure compounds have been evaluated for inhibitory activities of HIF-1 activation. Initial results have shown that purified natural products from marine organisms and plants inhibit HIF-1 activation. The chemical structures of some active compounds differ from that of the known flavanoid HIF-1 inhibitors. Inhibition of HIF-1 activation is associated with reduced hypoxic induction of HIF-1 target gene VEGF, a key angiogenic factor that promotes tumor angiogenesis.

The biochemical diversity represented by natural products produced by plants and marine organisms offer new and structurally diverse prototypes for the discovery of novel inhibitors of HIF-1 activation. These inhibitors of HIF-1 activation represent potential drug leads that target hypoxic tumor cells through blocking activation of survival genes.

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SYNTHESIS OF NEW INHIBITORS OF ALL-TRANS-RETINOIC ACID METABOLISM AND THEIR EFFECTS ON PROLIFERATION OF HUMAN BREAST CANCER CELLS

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A major reason for the clinical failure of all-*trans*-retinoic acid (ATRA) cancer therapy is the prompt emergence of resistance, believed to be caused by increased ATRA metabolism. Inhibitors of ATRA metabolism [also referred to as retinoic acid metabolism blocking agents (RAMBAs)] may therefore prove valuable in the treatment of cancers, including breast cancer. Thus, the potential mechanism of anti-tumor activity of RAMBAs might be linked to an increase in endogenous plasma and/or intracellular ATRA, modulating the proliferative, differentiation and/or apoptotic status of the tumor.

Novel C4-substituted retinoic acid analogs have been designed and synthesized and have been shown to be powerful inhibitors of hamster microsomal ATRA 4-hydroxylase enzyme(s). Our most potent RAMBA, VN/50A-1, with an IC_{50} value of 0.025 nM is 240,000-fold more potent than liarozole-fumarate, a previously identified inhibitor of ATRA metabolism. VN/12-1 and VN/50A-1 each at 1μM, inhibited completely (~ 100%) the metabolism of ATRA in intact MCF-7 human breast cancer cells.

The effects of some of our new RAMBAs alone or in combination with ATRA on proliferation of two human breast cancer cell lines, MCF-7 and MDA-MB-231 were examined. Cells were incubated with doses of ATRA and/or RAMBAs for 9 days and cell numbers were counted using a Coulter counter. Proliferation of MCF-7 cells was inhibited by ATRA in a concentration-dependent manner with a calculated IC_{50} values of 1.9 μM. In contrast, ATRA (up to 10μM) showed no effect on the proliferation of MDA-MB-231 cells. When MCF-7 cells were incubated with a combination of ATRA (100 nM) and liarozole-fumarate or our RAMBAs, the antiproliferative effect of ATRA was enhanced. Liarozole inhibited proliferation by 23.0% at 1 nM compared to control. However, all of our RAMBAs tested inhibited proliferation more potently than liarozole. VN/12-1 and VN/13-1, each at 1nM were the most potent, inhibiting cell proliferation by 75.0% and 72.8%, respectively. The enhancement by our RAMBAs of the antiproliferative effects of ATRA on MCF-7 breast cancer cells is probably due to inhibition of intracellular ATRA metabolism. Studies to identify a RAMBA with the best combination of high potency, low toxicity and favorable pharmacokinetic parameters for further preclinical anti-tumor evaluation are in progress.

In conclusion, our results have identified a number of potent RAMBAs that are potential new agents for the treatment of breast cancer.

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A UNIQUE TYPE II TOPOISOMERASE MUTANT THAT IS HYPERSENSITIVE TO A BROAD RANGE OF CLEAVAGE-INDUCING ANTITUMOR AGENTS

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The overall goal of this research is to understand the detailed mechanism of action of antitumor drugs that target type II topoisomerases. Bacteriophage T4 provides a useful model system for the study of these antitumor agents. Previous analysis showed that a drug resistant bacteriophage T4 mutant harbored two amino acid substitutions (S79F, G269V) in topoisomerase subunit gp52. When both mutations are present, the G269V substitution is thought to suppress a topoisomerase negative phenotype caused by the S79F substitution alone. Surprisingly, the single amino acid substitution, G269V, was shown to confer hypersensitivity *in vivo* to *m*-AMSA and oxolinic acid [Freudenreich C.H. et al., (1998) *Cancer Research* **58**, 1260-1267].

We purified the G269V mutant enzyme from *E. coli* cells infected with a topoisomerase overproduction strain of T4. Using DNA cleavage assays, we found the G269V enzyme to be hypersensitive to a number of cleavage-inducing inhibitors including *m*-AMSA, VP-16, mitoxantrone, ellipticine and oxolinic acid. While the mutant enzyme did not exhibit altered DNA cleavage site specificity compared to the wild-type enzyme, it did display an apparent 10-fold increase in drug-independent DNA cleavage. This suggests a novel mechanism of altered drug sensitivity in which the enzyme equilibrium has been shifted to favor the cleavage complex, resulting in an increase in the concentration of cleavage intermediates available to inhibitors.

It is unclear how the G269V substitution suppresses the topoisomerase negative phenotype displayed by the S79F mutant strain. Therefore, we have also purified the S79F single and S79F/G269V double mutant enzymes and are in the process of characterizing the defect. Understanding the interplay between these two positions and the nature of suppression could shed new light on enzyme function and the mechanism of hypersensitivity.

We believe that the G269V mutant defines a new category of type II topoisomerase mutants, namely those that are hypersensitive to all inhibitors that stabilize the cleavage complex. This unique class of topoisomerase mutants has never been characterized and may offer new insights into the mechanism of drug action. Additionally, understanding this hypersensitive class of enzymes may help in the design of more effective and less toxic drugs.

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MEASUREMENT OF THE ELECTRON DENSITY DISTRIBUTION OF ESTROGENS - A FIRST STEP TO ADVANCED DRUG DESIGN

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The scientific approach to preventing or curing disease requires understanding the mechanism by which the disease state is initiated or propogated. A significant body of knowledge exists to indicate that a large number of breast cancers are initiated by estrogens - the family of female hormones. It has been hypothesized that the mechanism involves first binding of the steroid molecule by the estrogen receptor in the nucleus of target breast cells, followed by secondary interaction which partially reorganizes the receptor pocket. This, in turn, modifies the surface of the protein such that the genes necessary for tumor development are activated. The purpose of the present study is to obtain information on the fundamental properties of estrogen molecules that are responsible for the binding event and for the subsequent reorganization of the receptor.

When two molecules approach one another, be they small organic molecules in solution or a steroid molecule approaching a protein, they sense each other's electrostatic potential. Much as two magnets will feel each other's magnetic field and orient themselves to maximize the attraction, so the two molecules will seek to align themselves in such a way as to approach negative regions to positive regions and vice versa. Perfect complementarity of the potentials will maximize the primary binding interaction and the secondary reorganization step necessary for gene expression. Molecules with different electrostatic potentials will have different binding affinities and different activities with respect to gene expression. These parameters are known for many compounds, whereas the electrostatic potentials are not.

The electrostatic potential is a complicated three dimensional function that is determined by the geometrical structure of a molecule and the distribution of the electrons and nuclei that make up that molecule. By using very accurate X-ray diffraction techniques, the complete description of the geometrical structure and the distribution of all of the electrons in a molecule may be determined. This information may then be used to calculate the electrostatic potential at any point in or around a molecule. We report the first studies of this type on a number of natural and unnatural estrogen derivatives.

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CELL-BASED SCREEN, ISOLATION, AND CHARACTERIZATION OF ANTIMITOTIC COMPOUNDS FROM NATURAL SOURCES

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Antimitotic agents kill cancer cells by causing arrest in mitosis. Those currently used in breast cancer therapy include the taxanes and the vinca alkaloids. Although valuable, these drugs have serious side effects, and many cancers become resistant to them. Our goal is to find new antimitotic drugs for the treatment of breast cancer using a novel cell-based assay to screen natural products and guide the purification of their active components.

The assay was used to screen 30,000 extracts of plants and marine organisms from our own collection and from the National Cancer Institute Natural Products Repository. We obtained 233 positive extracts. The fractionation, dereplication, purification and chemical structure elucidation of active compounds is ongoing but has been completed for 15 positive extracts, yielding new analogs of eleutherobin, rhizoxin, paclitaxel and okadaic acid, and three flavonoids, as well as compounds not previously described such as 13-hydroxy-15-oxozoapatlin.

The mechanism of action of the purified compounds has been studied. Most interact directly with tubulin but some, including 13-hydroxy-15-oxozoapatlin, have novel mechanisms of action currently under investigation. Our discovery of eleutherobin and several analogs in a widespread octocoral has permitted an extensive structure-activity study and the determination of the solution and crystal structures of eleutherobin. These have revealed unanticipated structural requirements for binding of microtubule-stabilizing agents to tubulin, which will be useful for the rational design of new microtubule-stabilizing drugs.

Synthetic schemes have been published for eleutherobin, but they are not practical and did not yield sufficient amounts for animal testing. We have been able to isolate from natural sources sufficient amounts to initiate studies of the therapeutic potential of this compound in experimental tumors in mice.

The cell-based screen is greatly facilitating the identification of new antimitotic agents. Testing of eleutherobin in experimental tumors will determine whether this class of microtubule-stabilizing compounds has potential for development into therapeutic agents.

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NOVEL BREAST TUMOR DIFFERENTIATION AGENTS

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The use of breast tumor differentiating agents to complement existing therapies has the potential to improve breast cancer treatment. We are investigating the use of antimalarials as breast tumor differentiation agents on the basis of previous work showing that quinidine induces differentiation and apoptosis in human breast tumor cells in vitro. To test whether antitumor activity was a general property of antimalarial agents, we screened a panel of quinidine analogs. We found that MCF-7 cell exit from the cell cycle into G0, marked by a loss of Ki67 antigen expression, was a typical response to antimalarial drugs. The rank order of potency for MCF-7 cell growth inhibition (MTS, IC50) in a series of antimalarials showed quinidine was the least potent among common antimalarial agents: primaquine (3 μM)<amodiaquin (7 μM)< halofantrine (10 μM)< chloroquine (35 μM)< quinine (40 μM) < quinidine (110 μM). These antimalarial agents also increased acetylated histone H4 levels in MCF-7 cells a response that is associated with differentiation in human breast tumor cells. We suggest that antimalarials raise histone H4 acetylation levels by a novel mechanism, because none of the antimalarials were direct inhibitors of HDAC 1/ 2 activity. Although all the antimalarials tested elicited two novel and significant antitumor responses in MCF-7 human breast tumor cells, histone H4 hyperacetylation and cell cycle exit into a non-proliferative (G0) state, only quinidine and chloroquine stimulated apoptosis in MCF-7 cells. To search for more potent structural analogs of chloroquine with similar antitumor responses in MCF-7 cells, approximately twenty amino alkyl substituted quinoline ring compounds from the NCI Compound Library were screened for growth inhibition (MTS IC50) and cell cycle exit (Ki67 index). NCI 4238 and NCI 10010 inhibited cell survival at sub-micromolar concentrations, stimulated exit from the cell cycle (Ki67 index), and promoted morphological changes typical of differentiating breast tumor cells. In summary, antimalarial agents and structural analogs exhibit antitumor properties in human breast cancer cells in vitro that are consistent with a novel mechanism of action as breast tumor cell differentiating agents.

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THE ROLE OF SYMBIOTIC BACTERIA IN THE BIOSYNTHESIS OF BRYOSTATINS: CLONING AND PRELIMINARY EXPRESSION OF A PUTATIVE BRYOSTATIN BIOSYNTHESIS PATHWAY FROM THE BACTERIAL SYMBIONT OF THE MARINE BRYOZOAN *BUGULA NERITINA*

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Bryostatins are biologically active complex polyketides that have been shown to exhibit activity against a wide variety of cancers, including breast cancer. The potential of these compounds as therapeutic agents is great; however, the availability of bryostatin limits research and development. Currently, these compounds are only found in trace amounts in the marine bryozoan Bugula neritina. Bryostatins are similar to bacterial secondary metabolites that are synthesized by modular type I polyketide synthases (PKS), and studies in our lab indicate that the biosynthetic source of the bryostatins is most likely B. neritina's bacterial symbiont, "Candidatus Endobugula sertula." Attempts to cultivate this symbiont are currently ongoing (see M. Haygood poster).

An alternative strategy for producing clinically useful quantities of the bioactive compound is to express the enzyme system responsible for their synthesis in a heterologous host. We have developed a protocol for extracting E. sertula enriched DNA from the bryozoan, screened this DNA for PKS genes, and have identified a large region of DNA with homology to the type I modular PKSs. Preliminary analysis shows that while several elements of this gene cluster are not "typical" for type I PKSs, the size and general domain content could encode a system capable of producing bryostatins. In addition, we have performed genomic Southern analysis on E. sertula enriched DNA, which suggests that the gene cluster we have identified is the only region with homology to PKS genes. We are currently working to express individual modules and genes from this region in heterologous systems. Future studies will include expression of all the modules of this PKS cluster. Ideally, these studies will lead to provision of a clinically useful supply of bryostatin for the treatment of breast cancer, as well as other cancers.

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NOVEL SMALL-MOLECULE INHIBITORS OF BCL-2 AS POTENTIAL NOVEL THERAPY FOR THE TREATMENT OF BREAST CANCER

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Bcl-2 is the founding member of a family of proteins, which includes both anti-apoptotic molecules such as Bcl-2 and Bcl-XL and pro-apoptotic molecules such as Bax, Bak, Bid and Bad. These molecules are crucial regulators of apoptosis. Bcl-2 protein is overexpressed in many forms of cancer, including breast, prostate, colorectal, leukemia and small cell lung cancer. Cancers that express high level of Bcl-2 are often resistant to chemotherapeutic agents or radiation therapy. Inhibition of the anti-apoptotic function of Bcl-2 thus represents a novel and promising strategy for overcoming the resistance of cancers to chemotherapy or radiation therapy and for developing an entirely new class of anticancer drugs.

We hypothesize that a small molecule that binds to the BH3 binding site in Bcl-2 may be capable of blocking the binding of Bcl-2 with pro-apoptotic members such as with Bad, Bak and Bax, and this blocking in turn may inhibit the anti-apoptotic function of Bcl-2 and induce apoptosis in breast cancer cells with Bcl-2 overexpression. Such small molecule inhibitors may have the great therapeutic potential for the treatment of breast cancer and many forms of cancer, in which Bcl-2 is overexpressed and for which traditional therapy has failed.

Through structure-based 3D-database searching of more than 250,000 small molecules, we have discovered a number of novel Bcl-2 small molecule inhibitors. Some of these small molecule inhibitors have sub-micromolar affinity in binding assays, and potently inhibit cell growth and induce apoptosis in breast cancer cells with Bcl-2 overexpression. Furthermore, we have showed that one potently small molecule inhibitor of Bcl-2 inhibits cancer growth in breast cancer MDA-MB-231 xenograft model, which has a high level of Bcl-2. Our long-term goal is to develop a small molecule inhibitor of Bcl-2 as a novel anti-cancer therapy for the treatment of breast cancer and other forms of cancer with high Bcl-2 expression, either alone or in combination with traditional therapy.

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STRUCTURE-BASED DESIGN OF ERBB-2 SELECTIVE SMALL-MOLECULE KINASE INHIBITORS

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Her-2/neu/erbB-2 gene encodes a 185 kD transmembrane glycoprotein that has partial homology with other members of the epidermal growth factor receptor (EGFR) family. ErbB-2 is overexpressed in 25-30% of breast cancers and it has been associated with a high risk of relapse and death. erbB-2 is a therapeutic target with clinically proven outcome for the treatment of breast cancer.

In breast cancers with erbB-2 overexpression, abnormal cell proliferation is caused by the extremely high tyrosine kinase activity and resulting high level of signal transduction. Theoretically, erbB-2 kinase inhibitors that are capable of entering cell, blocking this extremely high tyrosine kinase activity and shutting-down the signal transduction pathway mediated by erbB-2 may be used as potential therapeutic agents for the treatment of breast cancer. Although a potent, erbB-2 specific small-molecule kinase inhibitor capable of entering the cell is likely to have a great therapeutic potential for the treatment of breast cancers, to date, very few potent and erbB-2 specific small-molecule kinase inhibitors are reported.

Through a powerful computerized structure-based 3D-database searching, we have discovered potent and selective erbB-2 small molecule kinase inhibitors. We have shown that one such lead compound potently inhibits the kinase activity of Her-2 both *in vitro* and *in vivo*, while it has little effect on the kinase activity of EGFR, displaying a selectivity more than 100-times between Her-2 and EGFR. Furthermore, this lead compound was shown to potently inhibit tumor growth in multiple human breast cancer xenograft models with high Her-2 overexpression, while having little effect on tumor growth in xenograft models with high level of EGFR but low level of Her-2. Through structure-based design and chemical modifications, we have also derived new analogues that display more potent activity than the original lead compound.

The discovery of novel, potent and selective erbB-2 kinase inhibitors represents the first but important step to develop such inhibitors as a novel therapy, alone or in combination with other therapies, for the treatment of breast cancer in near future.

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THE SCHWEINFURTHINS: NEW LEADS FOR BREAST CANCER THERAPY

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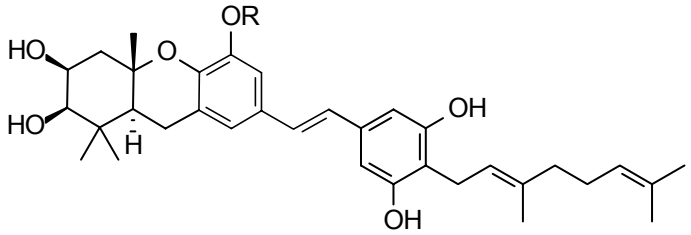
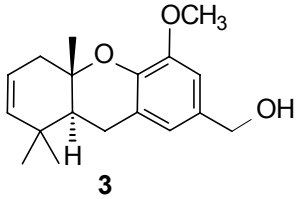
In 1998, three novel compounds were isolated from a plant extract as part of the National Cancer Institute's search for potential anticancer agents from natural sources. Two of these compounds, schweinfurthin A (**1**) and B (**2**), demonstrated a unique pattern of biological activity in assays against human cancer cell lines, which implies that they act in a way different from any current clinical agent. The combination of significant activity and a unique mode of action represents a promising lead for development of a new class of drugs for breast cancer therapy.

1 R = H (Schweinfurthin A)
2 R = CH_3 (Schweinfurthin B)



A major step in harnessing this biological activity would be to determine its cellular basis. While methods for determining the cellular targets of drugs are available, they require preparation of chemical derivatives of the parent compound. Because the natural products were isolated in very small amounts, and because reisolation from the natural source has proven unreliable, a chemical synthesis will be needed. We have undertaken this effort and completed chemical synthesis of two compounds corresponding to the left (**3**) and right (**4**) "halves" of Schweinfurthin B. Compound **3** was prepared through a 12-step sequence from vanillin, and still must undergo further elaboration to introduce the diol functionality. Compound **4** represents the entire "right" half of the natural product, and has been prepared through a 9-step sequence from 3,5-dihydroxybenzoic acid. Joining of these two halves is the next objective.



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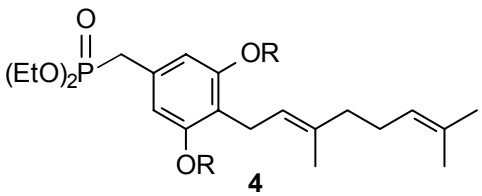

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We now have developed laboratory syntheses of two advanced intermediates that will be used to complete a chemical synthesis of the natural product Schweinfurthin B. Once synthesis of the natural product is complete, this material can be used in biological studies to better delineate the activity of the Schweinfurthins against breast cancers, and parallel synthesis can be conducted to provide probes that will illuminate the mechanism responsible for the anticancer activity. Ultimately, this research may allow development of a new class of drugs for breast cancer therapy.

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